Published in final edited form as:

Curr Mol Biol Rep. 2018 December ; 4(4): 180–190. doi:10.1007/s40610-018-0108-8.

Cellular senescence in intervertebral disc aging and degeneration

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Abstract

Purpose: Age is a major risk factor for multiple disease pathologies, including chronic back pain, which stems from age-related degenerative changes to intervertebral disc tissue. Growing evidence suggest that the change in phenotype of disc cells to a senescent phenotype may be one of the major driving forces of age-associated disc degeneration. This review discusses the known stressors that promote development of senescence in disc tissue and the underlying molecular mechanisms disc cells adopt to enable their transition to a senescent phenotype.

Recent findings: Increased number of senescent cells have been observed with advancing age and degeneration in disc tissue. Additionally, *in vitro* studies have confirmed the catabolic nature of stress-induced senescent disc cells. Several factors have been shown to establish senescence via multiple different underlying mechanisms.

Summary: Cellular senescence can serve as a therapeutic target to combat age-associated disc degeneration. However, whether the different stressors utilizing different signaling networks establish different kinds of senescent types in disc cells is currently unknown and warrants further investigation.

Keywords

Aging; intervertebral disc degeneration; cellular senescence

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Conflict of Interest

Prashanti Patil, Joon Lee, Gwendolyn Sowa, and Nam Vo each declare no potential conflicts of interest.

Laura J. Niedernhofer is an SAB member for Innate Biologics and Castle Creek, and reports a patent pending.

Paul D. Robbins is an SAB member for Innate Biologics, Tissuegene, Unicyte, Engene, and Genascence and co-founder of Genascence. Dr. Robbins also reports a patent pending.

Human and Animal Rights and Informed Consent

This article does not contain any studies with human or animal subjects performed by any of the authors.

Introduction:

The rising tide of silver tsunami, a moniker for population ageing, threatens to swamp our health-care system and impose heavy social and economic burdens. People are living longer than ever before due to advances in medicine, improved sanitation, control of infectious disease, and nutrition. For the first time in the history of mankind, the number of people age over 65 will soon outnumber children younger than 5 years of age [22]. However, due to the achievement in extending lifespan, people are now living with multiple age-related chronic diseases [85]. Therefore, it is imperative that we understand the biology of aging and develop therapeutic approaches to target the fundamental factors underlying age-related diseases.

Low back pain stemming from age-related degeneration of the intervertebral disc is one of the largest chronic disorders. It is the most common cause of joint-related chronic disability and debilitating pain in the elderly [91, 23]. Preserving healthy joints, particularly intervertebral discs in spine, is essential as decreased mobility is a validated predictor of loss of independence and mortality in the elderly [41, 16]. Individuals over 60 years old have a significantly higher risk of developing pain stemming from intervertebral disc degeneration (IDD). As such, there is now great impetus to understand what drives IDD with aging in order to preserve mobility and fitness in the elderly population.

The intervertebral disc (IVD) consists of the outer fibrous annulus fibrosus (AF) circumferentially enclosing the central gelatinous nucleus pulposus (NP). The NP serves as a shock absorber that dissipates the compressive forces towards the surrounding AF. The AF serves to bear tensile forces generated during bending or stretching. IVDs undergo age related degenerative changes earlier in life compared to other body organs [69, 10]. The age-related changes in the AF are characterized by loss of organized fibrous meshwork, an increase in the number and size of fissures, and an invasion of blood vessels along tears and clefts. With age, the NP transforms from a clear jelly-like substance into a fibrous tissue as a result of increased fragmentation and loss of proteoglycan (PG) in the extracellular matrix (ECM), with a concomitant decrease in water content, leading to tissue fissures and decrease in disc height [69, 84, 3]. The loss of the major disc PG, aggrecan, results in reduced capacity to resist compressive forces and is a hallmark of IDD. These age-associated changes compromise the structural integrity and biomechanical function of disc tissue.

The aberrant tissue homeostasis and eventual structural and functional decline of disc tissue with aging results from time-dependent accumulation of molecular and cellular damage. Numerous types of damage are implicated in driving disc aging, including accumulation of damaged proteins, mitochondrial damage and dysfunction, telomere shortening, DNA damage, and attrition of quality control mechanisms (autophagy, DNA repair, etc.) [77, 39, 36, 28]. One of the consequences of these different types of damage is that these disc cells develop a cellular senescence phenotype.

Cellular senescence is an evolutionarily-conserved cell fate of stable replicative arrest induced by different stressors, leading to loss of proliferative potential of normally replication-competent cells, resistance to cell death through apoptosis and generally

increased metabolic activity [12]. Senescent cells frequently develop a senescence-associated secretory phenotype (SASP) characterized by increased secretion of pro-inflammatory cytokines and chemokines, tissue-damaging proteases, factors that can impact stem and progenitor cell function, hemostatic factors, and growth factors [21]. Markers of senescent cells include increases in expression of the cell cycle regulators p16^{INK4A} and p21^{Cip1}, SASP factors such as interleukins 6 and 8 (IL-6, IL-8), monocyte chemoattractant protein-1 (Mcp-1), plasminogen-activated inhibitor-1 (Pai-1), senescence-associated β -galactosidase (SA- β gal), and telomere-associated and non-telomeric DNA damage foci [20].

It is thought that cell senescence evolved as a mechanism to suppress tumor growth following oncogene-mediated activation of cell division, where cell proliferation is halted and secretion of SASP factors stimulates the immune system to clear the senescent, pre-cancerous cells [12]. In addition to tumor suppression through oncogene induced senescence (OIS), senescence is driven by different types of stress including, mitochondrial dysfunction, telomeric erosion, persistent DNA damage, oxidative stress, and inflammation [20, 101]. Expression of SASP by senescent cells can act in an autocrine manner to reinforce senescence and via paracrine signaling to induce senescent establishment in neighboring cells. As a consequence, the health and function of the tissue undergo progressive deterioration unless the senescent cells are cleared. Indeed, most tissues of humans, primates, and rodents accumulate senescent cells with age [68, 40, 98]. Furthermore, senescent cells can be found in affected tissues in patients with age-related disorders such as osteoarthritis, chronic kidney diseases, and type 2 diabetes [13]. The use of genetic and pharmacologic approaches to reduce the burden of age-dependent cellular senescence leads to improved healthspan and lifespan, demonstrating the key role of senescent cells in driving aging [7, 17].

In this review, we focus on discussing the impact of biological stress known to be prevalent during IDD on the development of disc cellular senescence and the underlying molecular mechanisms that contribute to inducing and maintaining the senescent phenotype in disc cells. Additionally, we discuss the therapeutic strategies to ameliorate age-related IDD by targeting newly discovered mechanisms of disc senescence.

I. Significance of cellular senescence in context of disc degeneration and aging.

Expression of the cell cycle arrest protein p16^{INK4a}, a well-known senescent marker, increases with age in discs of patients and is positively correlated with expression of A Disintegrin And Metalloproteinase with Thrombospondin Motifs (ADAMTS)-5, and matrix metalloproteinases MMP-13 [59]. In contrast, there is a significant negative correlation between age and the extent of proliferating cells in human disc tissue. The increase in the fraction of disc cells stained with SA- β Gal positively correlates to degeneration grades of disc tissue and, more importantly, negatively correlates with Ki67 positive proliferating cells [31]. Hence, age-induced senescence in disc cells greatly reduces the percent of proliferative and functional cells in the disc.

Another mechanism through which senescent cells can contribute to the functional decline of disc tissue is by secretion of pro-inflammatory cytokines, including TNF α , IL-1 β , IL-17, IL-6, COX-2, which can 1) mediate secretion of matrix proteases in non-senescent disc cells; 2) promote bystander senescence; and 3) promote infiltration of immune cells, consequently reinforcing the inflammatory microenvironment and contributing to decreased matrix production and enhanced matrix protein degradation. Senescent disc cells undergo a phenotypic shift that disrupts the balance between disc ECM anabolism and catabolism. As a result, disc degeneration is accelerated. However, exactly what induces disc cell senescence with age and how senescence then drives pathology in the etiology of IDD is still unclear.

II. Mechanisms of intervertebral disc senescence.

What triggers senescence in disc cells? Various causes of cellular senescence have been identified that could drive disc senescence with age, including DNA damage, and mechanical, inflammatory, oxidative and nutritional stress. One or more of these could contribute to senescence in the disc and IDD.

Oxidative stress.

A substantial portion of the degenerative changes that occur in disc with aging are thought to be driven by oxidative stress. There is now ample evidence of increased oxidative stress in disc aging and degeneration. Malondialdehyde (MDA), a secondary product of peroxidation of polyunsaturated fatty acid residues, was found to be significantly higher in the discs of geriatric rats compared to those in adult rats [44]. Accumulation of intermolecular cross-links in long lived proteins such as collagen, a result of oxidative reactions, with age in disc is also well documented. One of the pathways for cross-link formation is via oxidative modification of glycosylated proteins, resulting in the formation of advanced glycation endproducts (AGEs) such as CML and pentosidine, both of which were found to accumulate with aging in human disc tissue [74, 90]. In particular, CML was found to be accentuated in degenerative region of disc tissue. Redox proteomic analysis revealed that the disc of aged mice contained higher levels of oxidized amino acids than discs of younger mice. Importantly, oxidized matrix proteins were found to be more susceptible to degradation by MMP 1,2, 9 and 13 proteases [86]. An increase in basement membrane collagen IV is seen peri-cellularly in cells that stain positive for CML in young adult discs. In older discs, however, an increase in hypertrophic collagen X is observed pericellularly in cells near clefts and tears. The expression of collagen IV and X is indicative of phenotypic alteration in disc cells as deposition of these collagen types is only seen in the basement membrane of epithelia of blood vessels or in the nerve sheath [74]. The exact phenotype of disc cells as a reaction to accumulative oxidative stress is still unknown. Numerous *in vitro* studies have shown that treatment of human and rat disc cells with oxidants such as hydrogen peroxide induces expression of matrix proteases and inflammatory cytokines with concomitant suppression in the expression of aggrecan and collagen matrix proteins. Importantly, abrogation in disc degenerative markers was seen in rat NP cells, a rabbit annulus puncture model, and a progeroid mouse model of disc degeneration upon treatment with antioxidants [73, 105, 106].

Oxidative stress is a well-known inducer of senescence phenotype. Since oxidative stress increases in disc with age, it likely contributes to the induction of senescence in the intervertebral disc. Indeed, human NP cells exposed to oxidative stress, e.g., hydrogen peroxide, become senescent and display PG homeostatic imbalance. These cells upregulate expression of p53 and p21 proteins and cease to proliferate [24, 76]. Additionally, H₂O₂-treated hNP cells show suppressed expression of aggrecan and collagen and enhanced expression of multiple proinflammatory cytokines (IL-6, IL-8, IL1 β , TNF α), and matrix proteases (MMP-1, 2, 3, 9, 13 and ADAMTS-4, 5). Consequently, increased fragmentation of aggrecan and collagen was also evident in H₂O₂-treated cells. Similarly, culturing NP cells in 20% oxygen upregulated ROS expression, induced DNA damage and cell cycle arrest, and elevated expression of catabolic factors- ADAMTS 4,5 and MMP 2,3, 14 [27]. Treatment of rat disc cells with exceptionally high glucose concentration was also reported to induce senescence via oxidative stress mechanism [79]. Together, these studies suggest that disc cells may undergo a phenotypic shift to a cellular senescence phenotype and promote the matrix homeostatic imbalance seen with aging in disc tissue in response to accumulative oxidative stress.

Genotoxic stress.

Chronic unrepaired DNA damage is a well-known driver of cellular senescence. Elevated cellular senescence and premature PG loss, similar to natural aging, were observed in discs of DNA repair-deficient *Ercc1*^{-/-} mouse model of accelerated aging [95]. In addition, adult mice chronically exposed to different types of genotoxic stress, including ionizing radiation and tobacco smoke, showed dramatic up regulation of disc p16^{INK4a}, a key marker of cellular senescence, and accelerated disc PG loss [71, 99]. In addition, primary disc cells isolated from *Ercc1*^{-/-} mice are mostly senescent and exhibit reduced capacity for PG synthesis and enhanced expression of MMPs. Hyperosmolality is another stimulus that is prevalent in disc tissue and has been reported to induce DNA double strand breaks and growth arrest in the G1 phase of the cell cycle [67]. NP cells are continuously exposed to hyperosmolality, up to 500 mOsm/kg H₂O in vivo as compared with <300mOsm/kg H₂O in the majority of the other tissues. Increased osmolality in NP cells was found to provoke chromatin changes and DNA damage. It is still unclear what level of hyperosmolality is needed to overwhelm the repair capacity of NP cells in order to induce DNA damage. Together, these observations strongly support the role of a role for DNA damage in inducing disc senescence. It is important to note that oxidative stress can induce oxidative DNA damage, which, in turn, leads to more oxidative stress.

Inflammatory stress.

The association between disc degeneration and inflammatory response has been extensively reported. Expression of the TNF receptors TNFR1 and TNFR2, TACE, interleukin-1 receptor antagonist (IL-1ra), and IL-1R1 have been reported in intervertebral disc and the expression of TNF α and IL-1 β has been shown to increase with severity of disc degeneration and aging in human and animal models [5, 60, 61]. Part of the inflammatory response in disc observed during degeneration and aging may be driven by the infiltrating immune cells like macrophages [83]. However, the resident disc cells are also capable of producing IL-6, IL-1 β and TNF α in the context of disc degeneration independent of

infiltrating leukocytes [38]. Moreover, significantly higher levels of IL-1 β , TNF α , IL-6 and IL-8 are found in patients with severe low back pain [54]. In addition, gene polymorphisms of IL-6 and IL-1 β are associated with increased risk of low back pain [51]. Importantly, the levels of the inflammatory mediators strongly correlate to IDD, indicating that they are likely causative of the disease. The inflammatory cytokines IL-1 β and TNF α promote expression of A ADAMTS-4, and -5, and matrix metalloproteinases MMP-1, -2, -3, -4, -13, and -14, and decrease expression of anabolic ECM proteins aggrecan and collagen II. In rat and bovine organ culture models, treatment with TNF α resulted in suppression of multiple collagen types, aggrecan, fibromodulin, increased expression of MMPs, ADAMTSs, pain-associated molecule nerve growth factor (NGF), and compromised disc biomechanics [81, 52]. *IL-1ra*^{-/-} mice, which lack the functional IL-1ra, an endogenous antagonist of IL-1R, display greater loss of PGs and collagens, and exhibit increased expression of MMPs 3 and 7 and ADAMTS4 in their IVD [80]. The discs of *IL-1ra*^{-/-} mice also had higher grade of histological degeneration and lower proliferative capacity compared to wild type mice.

It is plausible that the chronic exposure to inflammatory stress induces or increases the percent of senescent cells in the disc. Exposure of bovine caudal IVDs to TNF α for 21 days resulted in increased aggrecan breakdown, upregulation of IL-6, MMP-3, and SA- β gal [82]. Interestingly, the degradative changes were persistent in IVDs even after a recovery period of 14 days following a 7-day exposure to TNF α . In addition, loss proliferative capacity was observed in disc cells isolated from *IL-1ra*^{-/-} mice. This could be due to senescence induction as IL-1 β has been shown to induce senescence in articular chondrocytes and fibroblasts. Moreover, the collagen proteolytic fragments generated as a consequence of cytokine-mediated stimulation of MMPs and ADAMTS expression induced premature senescence in rat NP cells [26]. Importantly, loss of T2 weighted signal was observed in rat spine upon injection of rat NP cells treated with the collagen MMP fragments. Interestingly, treatment of aged rat AF cells with IL-1 β and the IVD of aged *IL-1ra*^{-/-} mice showed greater susceptibility to degenerative changes compared to young rat AF cells and young *IL-1ra*^{-/-} mice IVD [65]. This suggests that the transition to senescence phenotype results from chronic exposure to inflammatory milieu and that progressive impairment will ensue unless the disc is cleared of the senescent cells.

Mechanical stress:

IVDs are mechanically active and continuously load bearing throughout life. With aging, there is PG loss which depressurizes the NP, leading to abnormal loading of both NP and AF tissues [70]. Abnormal loading has been well-documented to promote degenerative changes in disc tissue [87]. These changes include reduced matrix synthesis, enhanced inflammatory, and catabolic effects. Whether chronic abnormal mechanical loading causes these changes by inducing cellular senescence is still not understood, however, a recent cell culture study reported that human NP cells exposed to high load undergo senescence [25].

Nutritional stress:

Intervertebral disc tissue is the largest avascular tissue in the body. The transport of oxygen, glucose, and other nutrients in the blood to the cells in the disc is largely dependent on diffusion from capillary bed in the vertebral bone adjacent to the endplate [96]. The

permeability of the endplate decreases with ageing; consequently, the flow of nutrients to the disc cells is reduced. This poor nutrition supply to the disc is thought to be an important contributor to the pathophysiology of disc degeneration. Indeed, reduced glucose and oxygen decreases the anabolic activity of disc cells in culture [9, 46]. Furthermore, increased risk of disc degeneration is seen in patients with clinical conditions associated with poor vascular function such as atherosclerosis and smoking [53, 89].

Serum components are critical regulators of cell cycle and numerous growth factors have been shown to inhibit apoptosis in disc cells including insulin-like growth factors (IGF)-1 and platelet derived growth factor (PDGF) [34]. Importantly, IGF-1 was shown to rescue rat AF cells from oxidative-stress induced premature senescence [32]. However, as the end plate becomes progressively impermeable to the flow of nutrients to disc, the limited nutrients may further enhance the progression to senescent phenotype of disc cells brought on by other stressors. Serum starvation of bovine IVD cells inhibits proliferative capacity and promotes the senescence phenotype [50]. In contrast, high serum concentration increases cell proliferation.

III. Signaling pathways in disc cellular senescence.

p53-p21^{Cip1}-Rb and p16^{INK4a}-Rb pathways are central to induction of senescence in disc cells.

p53-p21^{Cip1}-Rb and p16^{INK4a}-Rb are the two well-known and extensively studied pathways in context of cell cycle control, tumor suppression, and cellular senescence [8]. p53 guards genomic integrity by regulating several cellular processes, including cell cycle arrest, DNA repair, apoptosis and senescence, resulting from various stress signals. Upon genomic damage induced by endogenous and exogenous stressors, the Ataxia-telangiectasia-mutated (ATM) protein undergoes autophosphorylation and activation. The active ATM protein phosphorylates p53 directly to activate it or indirectly via phosphorylating Chk1 and Chk2 proteins. γ H2AX, a variant of the H2A histone protein family and a component of the histone octamer in nucleosomes, is another downstream target of ATM and is activated by phosphorylation by ATM. The phosphorylated γ H2AX serves to recruit and repair proteins to the DNA damage site. Persistent DNA damage response (DDR) is needed to maintain the cellular senescence phenotype. p53 also stimulates expression of the cyclin-dependent kinase inhibitor (CDK1) p21^{Cip1}, which prevents CDK2-mediated phosphorylation of Rb. Consequently, the cell cycle progression from G1 to S is inhibited as the hypo-phosphorylated Rb blocks the transcriptional activity of the E2F factor to promote the expression of the genes necessary for G1 to S progression. Similarly, expression of the CDKI p16^{INK4a} is increased by numerous cell stressors. p16^{INK4a} antagonizes the activation and activity of CDK/6, which leads to hypo-phosphorylation of Rb, inhibition of E2F and arrest of cell cycle.

Activation of both p53-p21^{Cip1} and p16^{INK4a}-Rb pathways were seen in cultures of human NP cells passaged serially to induce replicative senescence [48]. Oxidative stress generated by treatment with hydrogen peroxide was seen to promote γ H2AX foci formation and upregulation of the ATM-chk1-p53-p21-Rb pathway leading to stress-induced senescence in human NP cells [24]. Conversely, activation of p16-Rb pathway, not the p53-p21^{Cip1}

pathway, was seen in young AF and old NP rat cells induced to senescence via high-glucose mediated oxidative stress [79]. Furthermore, elevated p16^{INK4a} and disc degenerative changes were seen in wildtype mice exposed to genotoxic stressors, including ionizing radiation and mechlorethamine (MEC), a cancer therapeutic agent. Similarly, an increase in p16^{INK4a} was observed in the disc of DNA repair deficient *Ercc1*^{-/-} mice [71]. Together, these studies suggest that p53-p21^{Cip1} and p16^{INK4a}-Rb pathways may act in concert or in parallel to induce senescence. The critical determinants in driving these senescence-associated pathways in the disc include the senescence stimuli, cell type (AF vs. NP), and source of the disc cells (human, rat, mice, bovine). Thus, the disc tissue may contain a mosaic of distinct subpopulation of cells that respond to different stressors to undergo p53-p21^{Cip1} and/or p16^{INK4a}-Rb mediated senescence.

Canonical NF- κ B pathway and MAPK signaling are essential in maintaining senescence and SASP.

NF- κ B is a transcription factor that is a central mediator of inflammation, stress, and damage. NF- κ B can exist as a homodimer or heterodimer, with p50-p65 heterodimer being the most common form, which controls expression of numerous NF- κ B target genes. Oxidative and genotoxic stress can induce activation of NF- κ B via an ATM/IKK-dependent pathway [6]. Extensive evidence support the association between chronic activation of NF- κ B and many age-related degenerative diseases, including musculoskeletal disorders such as muscular dystrophy, osteoarthritis, and osteoporosis [1, 55, 2]. NF- κ B is also associated with age-associated IDD [102]. In disc tissue, activation of NF- κ B was seen to correlate with accumulative oxidative stress and increased with aging and degeneration [75]. Genetic reduction in the RelA/p65 subunit of NF- κ B or pharmacologic inhibition of the kinase activating NF- κ B (IKK) was shown to ameliorate several age-related degenerative changes in disc tissue [72]. Furthermore, increased NF- κ B transcriptional activity has been found in degenerative discs [102]. Higher levels of pro-inflammatory cytokines that are considered typical NF- κ B target genes, e.g. TNF- α , IL-1 β , IL-6 and IL-8 were present in symptomatic discs compared to asymptomatic discs [96]. Together, these findings support the role of dysregulated NF- κ B chronic activation in promoting IDD and age-related IDD.

NF- κ B also serves as a master regulator of several SASP factors, which in turn are needed to reinforce growth arrest in senescent cells. An increase in phospho-RelA/p65, mediated by IKK, was seen in TNF α -induced senescence in rat NP cells *in vitro* [62]. Similarly, treatment with hydrogen peroxide resulted in phosphorylation and activation of p65 subunit of NF- κ B, leading to increased NF- κ B transcriptional activity, increased expression of MMP-1, -2, -9, ADAMTS-5, and greater senescence in human NP cells [24]. In addition, the catabolic and protease expression of high oxygen tension mediated senescence of rat NP cells was abrogated by inhibition of NF- κ B activation [27]. Together, these studies suggest that NF- κ B activation accompanies and contributes to the development of SASP in disc cells.

Mitogen-Activated Protein Kinases (MAPKs) are a family of signal transduction pathways that allow the cells to respond to multiple extracellular inputs, such as hormones, growth factors, inflammatory cytokines, and environmental stresses such as ionizing radiation or

osmotic stress [45, 57]. In mammals, these diverse signals activate at least three major subfamilies of MAPKs, the extracellular signal-regulated kinases (ERK), c-Jun NH2-terminal kinases (JNKs), and p38 isoforms (p38MAPKs) [97, 11]. Activation of MEK/ERK and JNK are involved in the induction of cellular senescence [63, 66]. p38 MAPK activation is a marker of senescence and plays a vital role in establishing SASP, which probably affects local tissue homeostasis [29]. Consistent with *in vitro* data, up-regulated p38 MAPK expression has been reported in senescent AF cells isolated by laser capture microdissection [33].

Increased disc cell proliferation and formation of cell clusters is a characteristic feature of disc degeneration, likely due to the over-expression of growth factors and their receptors [50]. Growth factors such as PDGF, IGF-I or bFGF can stimulate cell proliferation via ERK activation. Conversely, in rat NP cells exposed to high oxygen tension, growth arrest and generation of SASP factors were seen to be dependent on ERK, JNK and p38 activation [27]. These studies suggest that MAPKs may be involved in the establishment of disc cellular senescence and function in degeneration and ageing of disc tissue.

SIRT1 suppresses cellular senescence and ameliorates disc degenerative changes.

SIRT1 is a well conserved nicotinamide (NAD⁺) dependent deacetylase and has been shown to be associated with multiple age-related pathologies, including several cancers and neurodegenerative disorders [15]. It regulates the life span of organisms such as yeast and flies [64, 92]. Recent studies implicate SIRT1 as a key longevity gene that plays a pivotal role in many cellular functions, including cell differentiation and proliferation, apoptosis, and cell aging. Previous studies have investigated the roles of SIRT1 in cell senescence. SIRT1 was shown to inhibit acetylation and activation of p53 in PML-induced senescence [58]. Expression of SIRT1 is severely reduced in degenerated discs [37]. However, treatment with resveratrol, a known activator of SIRT1, suppresses apoptosis, p16^{INK4a} and p21^{Cip1} expression along with dampening of matrix proteases (MMP 13 and ADAMTS-5) in human NP cells isolated from degenerated discs [37]. Cartilage endplates (CEP), hyaline cartilage which separates nucleus pulposus from vertebral end plates, obtained from patients with degenerative discs had lower levels of SIRT1 positive cells compared to CEP from patients with lower lumbar fracture [107]. The lower expression of SIRT1 was accompanied by elevated levels of p53 and p21^{Cip1} in CEP of patients with disc degeneration. SIRT1 overexpression in end-plate disc cells abrogated the H₂O₂-stress induced senescent growth arrest by dampening the expression of p53 and p21^{Cip1}. Additionally, genetic ablation of an allele of SIRT1 in mice was shown to suppress collagen and increase p16^{INK4a} protein levels in coccygeal disc of mice [103]. Similarly, in mice with disc degeneration induced by coccygeal disc puncture, injection of resveratrol was seen to ameliorate collagen suppression and p16^{INK4a} elevation. These results suggest that stimulating SIRT1 activity by either increasing the level of the NAD⁺ co-factor by treatment with nicotinamide mononucleoside (NMN) or nicotinamide riboside (NR), or stimulating SIRT1 directly with resveratrol or related compounds, could potentially suppress disc senescence and related pathologies.

Wnt/ β -catenin mediates development of senescent phenotype in disc cells.

The Wnt/ β -catenin pathway is involved in a wide range of cellular processes, including cell migration, differentiation, proliferation, and in pathological processes like tumorigenesis [19]. Several reports have documented the crucial role of Wnt/ β -catenin signaling in chondrocyte differentiation, and cartilage development and function. However, its relevance in the context of intervertebral disc degeneration and ageing is not well understood. Wnt/ β -catenin signaling is upregulated during disc degeneration, possibly mediated by lncRNA H19 [100]. The expression of H19 and Wnt are increased during disc degeneration and knockdown of H19 suppresses the expression of Wnt target proteins cyclin D1 and Myc. It was shown in the same study that overexpression of H19 in NP cells induces growth arrest, upregulates matrix protease synthesis and dampens matrix production. This suggests that lncRNA regulated Wnt signaling could contribute to the development of senescence in disc cells. This result was surprising as Wnt signaling was seen to decrease with ageing in the lumbar disc of mice [43]. It is possible that the levels of Wnt protein and its target genes increase only in senescent disc cells, but decrease in other non-senescent disc cells. In accordance with this, activation of Wnt/ β -catenin signaling upon exposure to LiCl was seen to modulate cellular senescence and expression of MMP in rat disc cells [42]. Further investigations are needed to elucidate the role of Wnt signaling in disc cellular senescence and aging-associated IDD.

IV. Potential therapeutics targeting senescence to treat age-related IDD.

Several underlying mechanisms have been shown to mediate cellular senescence in disc cells. The various signaling pathways serve as potential therapeutic targets to ameliorate not only senescence development, but also age-related disc degenerative changes. Metformin, a glucophage, inhibits complex I of ETC and can therefore suppress production of mitochondrially derived reactive oxygen species (ROS), a major contributor to disc degeneration [94]. Tert-butyl hydroxide induced senescence in NP cells was suppressed when treated with metformin. More importantly, metformin treatment was able to ameliorate IDD in a puncture-induced rat model [18]. Another promising target is the NF- κ B pathway since, as discussed above, its chronic activation has been closely linked to age-related diseases. Previous studies demonstrated that blocking NF- κ B activity pharmacologically and genetically in the *Ercc1*^{-/-} rodent model of accelerated aging delay the onset of age-dependent disc proteoglycan loss and other degenerative changes [72]. Moreover, intra-discal injection of 'naked' NF- κ B decoy oligonucleotides proved effective in partially restoring disc height in an animal model of IDD [78].

It was recently shown that drugs able to specifically kill senescent cells, termed senolytics, can improve aged disc pathology. In particular, the senolytic combination of dasatinib and quercetin was shown to not only reduce senescence and improve multiple age-related pathologies, but also improve PG content in the disc of progeroid *Ercc1*^{-/-} mice [108]. Similarly, treatment of *Ercc1*^{-/-} mice with the senolytic HSP90 inhibitor 17-DMAG resulted in not only an extension of healthspan, but also improved PG content [30]. Furthermore, intra-articular injection of the senolytic drug Navitoclax, a Bcl-2 family inhibitor, was recently shown to slow the progression of age-associated and post-traumatic osteoarthritis in

aged mice [47]. However, the effect of these senolytics on disc pathology in naturally aging mice is unknown.

V. Conclusions and Perspectives.

Growing evidence implicates cellular senescence as a driver of age-related IDD, but research on disc cellular senescence is still in its infancy. The initiating stressors, the mediating signaling pathways, and the resulting phenotype underlying cellular senescence in IVD are only beginning to come to light with in vitro and in vivo experiments; the exact mechanisms of each of these processes are still poorly understood. For instance, while it is clear from research using the DNA repair-deficient *Ercc1*^{-/-} mice that persistent DNA damage drives disc cellular senescence, it is still far from clear what drives DNA damage and senescence in disc cells during the course of natural aging. More research is needed to determine the relative contributions of the different stressors (inflammatory, oxidative, mechanical, and nutritional stress) in promoting cellular senescence in IVD tissue. Likewise, little is known about the roles of the different signaling networks (MAPK, NF- κ B, SIRT1, and Wnt/B-catenin) in mediating the establishment of disc cellular senescence. Whether different stressors trigger different signaling pathways to establish different types of disc senescence phenotypes is an important research area that has not been investigated. In addition, aging is systemic which raises the question of whether cellular senescence in neighboring and distal tissues influence age-related IDD. Answering these questions is necessary to provide the basic information needed for the development of effective therapies to target cellular senescence to treat age-related IDD. This is imperative given a rapidly growing aged population, which will bring a sizeable burden of IDD-associated chronic pain and disability.

Acknowledgements.

This work was made possible in part by the Public Health Service grants R01 AG044376-01 (NV), P01-AG043376 (LJN and PDR) and U19 AG056278 (LJN and PDR) from the National Institute of Health, the Glenn Foundation (LJN) and UPMC Department of Orthopaedic Surgery. The authors thank Jessa Darwin for her editorial assistance.

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Table 1:

Cellular senescence-related research on intervertebral discs

Models	Treatments	Senescence Markers	Disc phenotypes	Ref
AGING				
<i>Ercc1^{-/-}</i> mice	<i>Accelerated aging due to DNA repair-deficiency</i>	Increased p16 ^{INK4A}	Early onset of disc PG loss	[95]
<i>Humans</i>	<i>Natural aging</i>	Increased p16 ^{INK4A} , SA- β -gal, telomere length shortening	Increased MMP13, ADAMTS5	[35, 59]
<i>Sand rats</i>	<i>Spontaneous accelerated aging</i>	SA- β -gal	Not reported	[35]
GENOTOXIC STRESS				
<i>In vivo models</i>				
<i>C57Bl/6</i> mice	<i>Mechloroe thamine</i>	Increased p16 ^{INK4A}	Early onset of disc PG loss	[71]
<i>C57Bl/6</i> mice	<i>Ionizing radiation</i>	Increased p16 ^{INK4A}	Early onset of disc PG loss	[71]
<i>In Vitro models</i>				
<i>hNP cell culture</i>	<i>Ionizing radiation</i>	IL-6,8, MMP-1,2,3,9	Not reported	[93]
<i>hNP cell culture</i>	<i>Hyperosmolar condition</i>	Increased p53, γ H2A.X	Not reported	[67]
OXIDATIVE STRESS				
<i>In vivo models</i>				
<i>C57Bl/6</i> mice	<i>Tobacco smoking</i>		Early onset of IDD	[71]
<i>Ercc1^{-/-}</i> mice	<i>Anti-oxidant XJB-5-131</i>		Blunt age-related IDD	[73]
<i>In Vitro models</i>				
<i>hNP cell culture</i>	<i>H₂O₂</i>	Increased p53, p21, p16 ^{INK4a} , SA- β -gal, γ H2A.X, MMPs	Decreased aggrecan mRNA, increased aggrecan fragmentation and PG synthesis	[76]
<i>hNP cell culture</i>	<i>21% O₂</i>	Increased p53, p21, p16 ^{INK4a} , SA- β -gal, γ H2A.X, MMPs	Not reported	[27]
INFLAMMATORY STRESS				
<i>In vivo models</i>				
<i>Ercc1^{-/-} ; p65^{+/-}</i> mice	<i>NF-κB knockdown</i>		Suppression of PG loss	[72]
<i>Ercc1^{-/-} ; mice</i>	<i>NBD peptide/NF-κB KD</i>		Early onset of disc PG loss	[72]
<i>IL-1α^{-/-}</i> mice	<i>IL-1 receptor antagonist (IL-1Ra) KD</i>	Decreased proliferation potential, increased IL-1 β , MMP-3,7, ADAMTS-4	Decreased disc height, loss of lamellae and cell number, increased ossification	[80]
<i>In Vitro models</i>				
<i>Ex vivo bovine disc</i>	<i>TNFα</i>	Increased SA- β Gal, IL-6, MMP-3	Decreased aggrecan and collagen I & II, increased aggrecan fragmentation	[84]
<i>Rat NP cell culture and ex vivo disc culture</i>	<i>TNFα</i>	Increased SA- β Gal, p53, p16, decreased telomerase activity	Decreased aggrecan and collagen II	[62]
MECHANICAL STRESS				
<i>In vivo models</i>				
<i>Sand Rats</i>	<i>Forelimb amputation, vertical loading</i>	Increased SA- β -gal, p16 ^{INK4a} and retinoblastoma	Not reported	[104]
<i>In Vitro models</i>				

Models	Treatments	Senescence Markers	Disc phenotypes	Ref
<i>Rat NP cell culture</i>	<i>Cyclic Mechanical Tension</i>	Increased p53, p21, p16 ^{INK4a} , SA-β-gal, γH2A.X	Not reported	[25]
NUTRITIONAL STRESS				
<i>In vivo models</i>				
<i>Rats</i>	<i>Diabetes</i>	Increased p16 ^{INK4a}	Decreased GAG, aggrecan, collagen I & II	[49]
<i>In Vitro models</i>				
<i>Rat AF and NP cell culture</i>	<i>Glucose</i>	Increased SA-β-gal, p53, p21, p16	Not reported	[79,56]